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Characterization of a Novel Composite Staphylococcal Cassette Chromosome *mec* (SCC*mec*-SCC*cad/ars/cop*) in the Neonatal Sepsis-Associated *Staphylococcus capitis* Pulsotype NRCS-A

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Multiresistant *Staphylococcus capitis* pulsotype NRCS-A has been reported to be a major pathogen causing nosocomial bacteremia in preterm infants. We report that the NRCS-A strain CR01 harbors a novel 60.9-kb composite staphylococcal cassette chromosome *mec* (SCC*mec*) element, composed of an SCC*mec* with strong homologies to *Staphylococcus aureus* ST398 SCC*mec* and of an SCC*cad/ars/cop* harboring resistance genes for cadmium, arsenic, and copper. Whole-genome-based comparisons of published *S. capitis* strains suggest that strain CR01 acquired the two elements independently.

Coagulase-negative staphylococci (CoNS) are common commensals of the human skin and mucosa that are now recognized as important opportunistic pathogens that cause nosocomial bloodstream and indwelling medical device-related infections (1). Recent studies have reported the emergence of methicillin-resistant, vancomycin-heteroresistant *Staphylococcus capitis* as an important cause of late-onset sepsis (LOS) in neonatal intensive care units (NICUs) in France and other countries (2–6). Surprisingly, all *S. capitis* strains from intensive care neonates were reported to belong to a single pulsed-field gel electrophoresis pulsotype, NRCS-A, and to harbor a type V-related staphylococcal cassette chromosome *mec* (SCC*mec*) element (6). In contrast, *S. capitis* bloodstream isolates from adult patients belong to several distinct, non-NRCS-A pulsotypes. To gain insights into the genetic characteristics of this LOS-specific multiresistant *S. capitis* clone, we sequenced and characterized the SCC*mec* element of one representative strain (CR01) with the NRCS-A pulsotype.

Strain CR01 was isolated from the blood cultures of an infant diagnosed with LOS in 2007 in the NICU of the Hospices Civils de Lyon, France. The strain was part of a previously published collection (6), had the pulsotype NRCS-A, and harbored a type V-related SCC*mec* element. We performed whole-genome pyrosequencing (454 Life Sciences/Roche) for this strain and used bioinformatics tools to identify and characterize a novel 60.9-kb composite SCC*mec* element (DDBJ/EMBL/GenBank accession no. KF049201). The cassette was found to be inserted into the characteristic 3′ end of the *orfX* gene (7, 8). To define the SCC region, we searched for SCC-specific insertion site sequences (ISSs) (9, 10). Three ISSs were found, with the furthest downstream from *orfX* being partially deleted. Because the predicted SCC region was found in two different contigs, the SCC sequence was closed using PCR.

SCC*mec* characterization and similarities to SCC*mec* elements of livestock-associated *Staphylococcus aureus*. A 38.8-kb SCC*mec* element was identified immediately downstream of *orfX*. This element harbored a type V (5C2&5) SCC*mec* and contained 42 open reading frames (ORFs; orf1 to orf42 in Table S1 in the supplemental material), as predicted using the syntactic and functional annotation implemented in the MicroScope/MaGe platform pipeline (11, 12). Using the MicroScope genome browser, the sequence of the CR01 SCC*mec* element was aligned with 22 genomes of methicillin-resistant *Staphylococcus* strains, which are available in the MaGe database. Two genomes in this database carried SCC*mec* elements showing a remarkable similarity to the NRCS-A strain CR01 SCC*mec* in terms of gene content, gene order, and nucleotide identity. Both SCC*mec* elements were carried by livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) strains, namely, the S0385 and 08BA02176 strains, which belong to the highly prevalent LA-MRSA sequence type 398 (ST398) (Fig. 1; see also Table S1). A detailed comparison of gene organization in these three SCC*mec* elements revealed that the structures of the joining regions J3 and J2 and the *mec* and *ccr* complexes were identical to those found in the ST398 LA-MRSA strain S0385, whereas the joining region J1 was identical to that of the ST398 LA-MRSA strain 08BA02176. In both the CR01 and the 08BA02176 SCC*mec* elements, the J1 region contained clustered regularly interspaced short palindromic repeat (CRISPR) loci with their associated *cas* genes. CRISPR loci are the sole prokaryotic defense mechanism against foreign DNA conferring an adaptive immune response, inversely to other defense mechanisms such as restriction-modification (R-M) systems (13). Using the online CRISPRFinder software tool (14), we identified two CRISPR elements in the NRCS-A strain CR01 located immedi-

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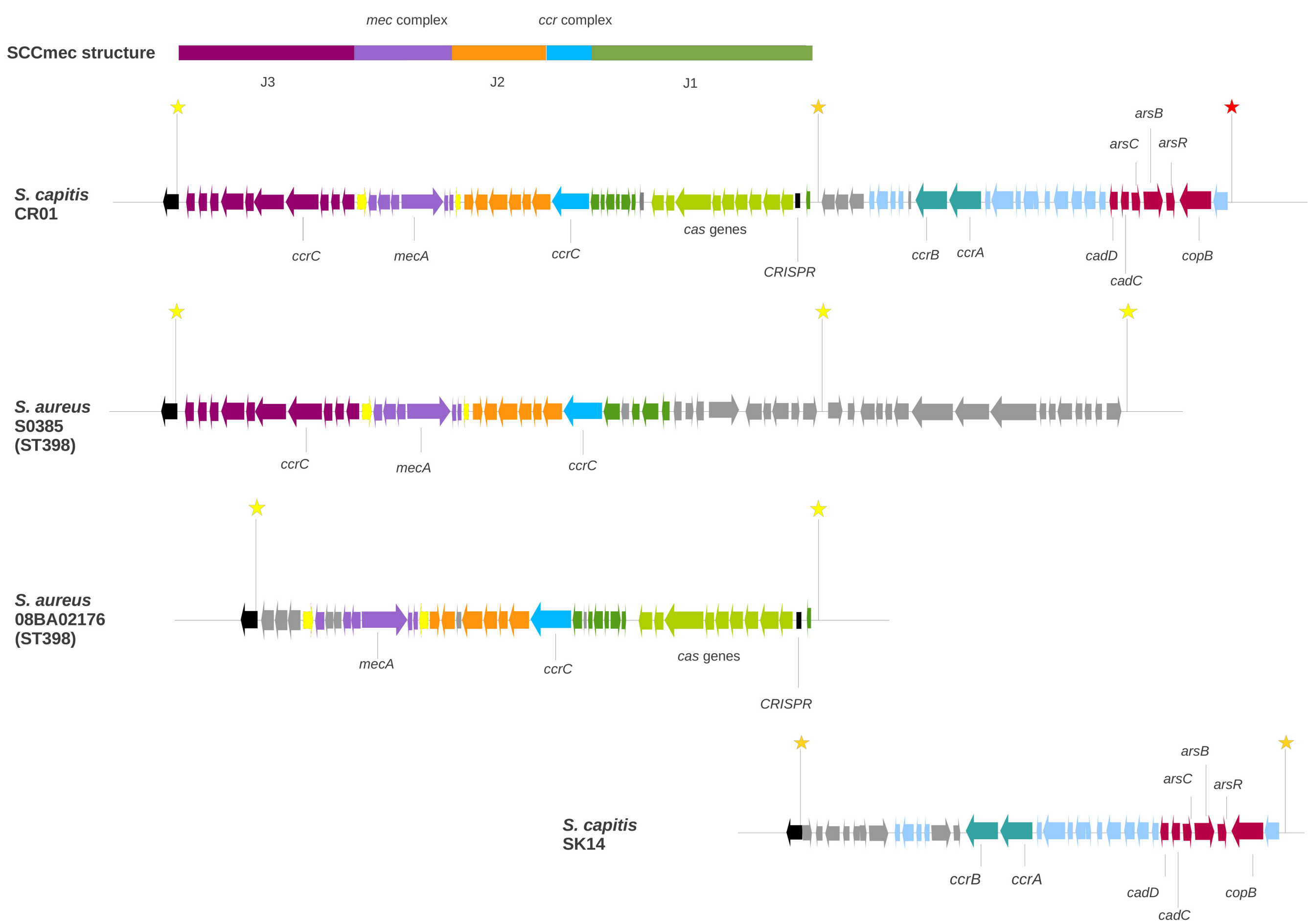

FIG 1 Comparative structure analysis of the composite staphylococcal cassette chromosome *mec* SCC*mec*-SCC*cad/asr/cop* element of *Staphylococcus capitis* strain CR01. The sequence of the SCC*mec*-SCC*cad/asr/cop* element of strain CR01 was aligned against whole genomes of ST398 *S. aureus* strains S0385 and 08BA02176 and of *S. capitis* strain SK14. Open reading frames (ORFs) are shown as arrows indicating the transcription direction and colored according to the SCC*mec* region to which they belong (J3, *mec* complex, J2, *ccr* complex, or J1). Homologous gene clusters in different strains have similar colors. The chromosomal *orfX* gene and the transposase IS*431* are represented by black and yellow arrows, respectively. Insertion site sequences (ISSs) are indicated by vertical lines and colored stars as follows: light yellow star, *ccrC* recombinase ISS; dark yellow star, *ccrAB* recombinase ISS with associated direct repeat (DR) sequences; red star, vestigial *ccrAB* ISS and DR sequences. The CRISPR-associated genes (*cas* genes) are indicated in light green within the J3 region, while the *ccr* complex in the SCC*cad/ars/cop* element is colored in turquoise and the resistance genes in dark red.

ately before the second ISS (Fig. 1). The first CRISPR element consisted of 16 direct repeats (DRs) and 15 spacers. The second CRISPR element consisted of three nonconserved DRs and two spacers. This subtype III-A CRISPR locus (15) (including the 2 CRISPRs and the *cas* genes) showed a high similarity to the element found in the ST398 LA-MRSA strain 08BA02176 SCC*mec* element (see Fig. S1 in the supplemental material).

To further investigate the relationships between the CR01 SCC*mec* elements and those of ST398 *S. aureus* (strains S0385 and 08BA02176), we characterized the SCC*mec*-associated direct repeat units (*dru*) using the scheme described by Goering et al., which has been described as a discriminant epidemiological tool for differentiating highly uniform epidemic methicillin-resistant strains (16). *dru* analysis showed that the SCC*mec* cassettes of both the CR01 strains and the ST398 08BA02176 strain shared the same *dru* type (5a-2d-4a-0-2d-5b-3a-2g-4b-4e-3e), namely, dt11c, which further highlighted the close genetic proximity between these SCC*mec* elements. Strain S0385 harbored a different *dru* type, namely, dt10a (dt10a, 5a-2d-4a-0-2d-5b-3a-2g-3b-4e), but presenting only one mutation in the ninth repeat and one repeat fewer. The *dru* type found in strain CR01 was identical to the ones found in other *S. capitis* NRCS-A strains ($n = 12$, data not shown), further corroborating the clonality within this lineage.

Collectively, these findings suggest that the *S. capitis* NRCS-A strain CR01 and ST398 LA-MRSA strains have undergone a recent horizontal transfer of SCC*mec* elements originating from a common source.

SCC*cad/ars/cop* characterization and relationships to other SCC elements. Immediately downstream of the SCC*mec* domain, we identified a second, 22.2-kb-long SCC domain (SCC*cad/ars/cop*) harboring 29 predicted ORFs (orf43 to orf70 in Table S1 in the supplemental material), including a type 8 *ccrA1B3* complex with 90% similarity to the *ccr* complex recently described in *S. aureus* strain LGA251 carrying a type IX SCC*mec* and the novel *mecC* allele (17). A comparison of the overall structure of the SCC*cad/ars/cop* with other SCC elements revealed only two regions with partial similarities to previously described SCC elements. The first region encompassed nine ORFs (orf47 to orf56 in

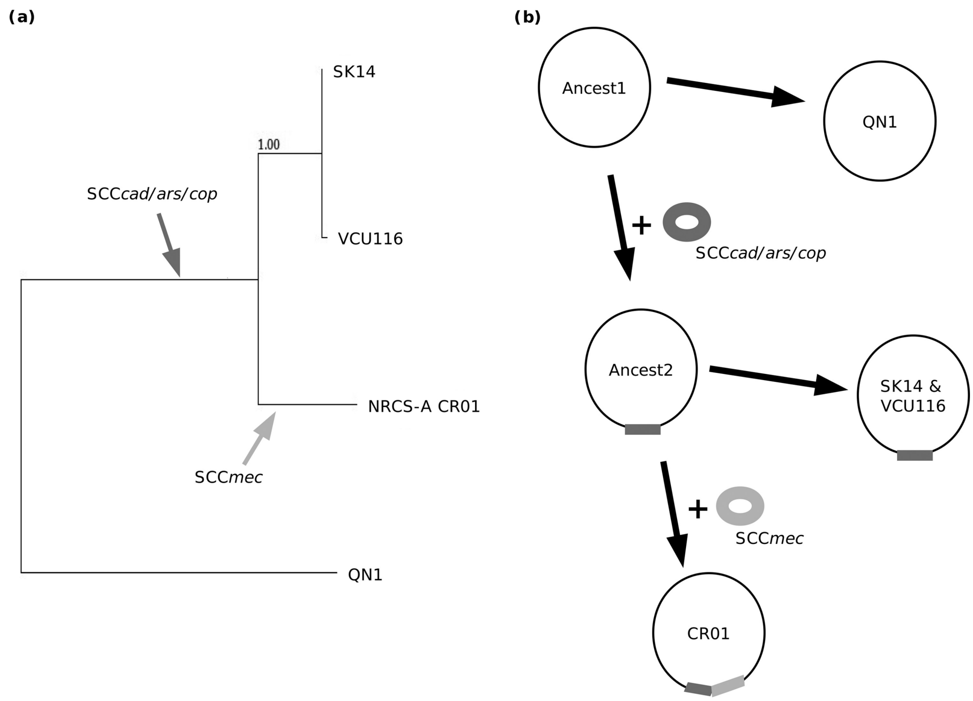

FIG 2 Phylogenetic analysis of whole-genome-sequenced *S. capitis* strains and proposed evolutionary history of SCC element acquisition in *S. capitis*. (a) SNP-based maximum likelihood tree of the 4 *S. capitis* whole-genome sequences. A total of 27,836 SNPs (indels excluded) were obtained by alignment against the reference strain *S. epidermidis* ATCC 12228. Numbers at the branching points represent the aLRT branch support score obtained. Proposed acquisition events of SCC*cad/ars/cop* and SCC*mec* elements are indicated by dark and light gray arrows, respectively. (b) Proposed model of sequential acquisition of SCC elements in the *S. capitis* clade: in a first event, the SCC*cad/ars/cop* element harboring multiple resistance genes to heavy metals was inserted via site-specific recombination at the end of the *orfX* gene; a second insertion occurred in the SCC*cad/ars/cop*-positive strain CR01 with the insertion of SCC*mec* at the 3′ end of *orfX*. Abbreviations: Ancest1, first ancestor; Ancest2, second ancestor (of CR01, SK14, and VCU116).

Table S1), including the *ccrA1B3* gene complex; this region had an organization similar to the regions found in the SCC*pbp4* element of the *Staphylococcus epidermidis* strain ATCC 12228 and in the type II SCC*mec* elements of the *S. aureus* strains MRSA252 and N315 (18). The second region encompassed 11 ORFs (orf58 to orf69 in Table S1) and included genes for the detoxification of heavy metals, namely, cadmium (*cadDC*), arsenic (*arsCBR*), and copper (*copB*). All 11 ORFs were highly homologous to those found in the J1 regions of the SCC*mec* element of CC398 MRSA *S. aureus* JCSC6943 (19) and of the ΨSCC*mec*(*h1435*) domain in *Staphylococcus haemolyticus* JCSC1435 (18), with nucleotide identities greater than 97.5%.

Of note, previously published NRCS-A strains have been reported to harbor only a type V-related SCC*mec* element (*ccrC* complex), as determined using the multiplex PCRs described by Kondo et al. (6, 20). To understand why these PCRs failed to detect the SCC*cad/ars/cop* type 8-related *ccrAB* complex, Kondo's primer sequences were aligned to the NRCS-A strain CR01 *ccrA1* and *ccrB3* alleles. Several mismatches were observed between the *ccrA* primers and their target sequence, which likely prevented amplification of the *ccrAB* complex and so explain the misinterpretation of Kondo's scheme.

To assess whether SCC elements are present in other *S. capitis* strains, we performed a BLAST search (http://blast.ncbi.nlm.nih.gov) of the composite SCC*mec*-SCC*cad/ars/cop* sequence against publicly available genomes from methicillin-susceptible *S. capitis* isolates, namely, those of strains QN1 (GenBank accession no. AJJH00000000) (21), SK14 (GenBank accession no. ACFR00000000), and VCU116 (GenBank accession no. AFTX00000000). None of these strains harbored an SCC*mec* element. Interestingly, whereas strain QN1 had an intact *orfX* gene, both the SK14 and the VCU116 strains carried homologous SCC*cad/ars/cop* elements inserted into the 3′ end of *orfX*. These latter elements were highly similar to the SCC*cad/ars/cop* found in the NRCS-A strain CR01 (Fig. 1; see also Table S1 in the supplemental material), except for the first seven ORFs after the insertion at the end of *orfX*. Gene organization and nucleotide sequences were identical in 22 remaining ORFs (orf47 to orf49 and orf53 to orf70 in Table S1).

Evolutionary history of SCC*cad/ars/cop* and SCC*mec* acquisition by *S. capitis* NRCS-A. To assess the phylogenetic relationships between the *S. capitis* strains CR01 (NRCS-A clone), QN1, SK14, and VCU116, we performed a whole-genome-based single nucleotide polymorphism (SNP) analysis and constructed a maximum likelihood (ML) SNP tree using the snpTree online web server (22) and PhyML (23), respectively (see the supplemental material). Interestingly, all SCC*cad/ars/cop*-positive strains clustered on a monophyletic clade, separate from the SCC-negative strain QN1. Furthermore, the sole SCC*mec*-positive strain (CR01) was the most divergent compared with strains SK14 and VCU116 (Fig. 2a). These results suggest the occurrence of at least two independent SCC acquisition events within the *S. capitis* species (Fig. 2b). The first acquisition of the SCC*cad/ars/cop* occurred in the ancestral lineage of strains CR01, SK14, and VCU116, which was followed by later acquisition of the SCC*mec* element in the ancestor lineage of the NRCS-A clone.

Conclusions. The highly clonal population of *S. capitis* NRCS-A, which is endemic in NICUs in several countries distant from one another (5), harbors a novel SCC*mec*-SCC*cad/ars/cop* composite island that has likely emerged from two independent acquisition events.

The high degree of structural similarity of the SCC*mec* type V element and the partial conservation of genes for heavy metal detoxification strongly suggest the occurrence of genetic shuffling involving *S. capitis* and CC398 MRSA strains. Whether SCC element exchanges occurred directly between these strains or indirectly via a common SCC element donor is presently unclear and warrants further investigation.

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